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Potential and limitations of on-line comprehensive reversed phase liquid chromatography × supercritical fluid chromatography for the separation of neutral compounds: An approach to separate an aqueous extract of bio-oil

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ABSTRACT

On-line comprehensive Reversed Phase Liquid Chromatography × Supercritical Fluid Chromatography (RPLCxSFC) was investigated for the separation of complex samples of neutral compounds. The presented approach aimed at overcoming the constraints involved by such a coupling. The search for suitable conditions (stationary phases, injection solvent, injection volume, design of interface) are discussed with a view of ensuring a good transfer of the compounds between both dimensions, thereby allowing high effective peak capacity in the second dimension. Instrumental aspects that are of prime importance in on-line 2D separations, were also tackled (dwell volume, extra column volume and detection). After extensive preliminary studies, an on-line RPLCxSFC separation of a bio-oil aqueous extract was carried out and compared to an on-line RPLCxRPLC separation of the same sample in terms of orthogonality, peak capacity and sensitivity. Both separations were achieved in 100 min. For this sample and in these optimized conditions, it is shown that RPLCxSFC (with Hypercarb and Acquity BEH-2EP as stationary phases in first and second dimension respectively) can generate a slightly higher peak capacity than RPLCxRPLC (with Hypercarb and Acquity CSH phenyl-hexyl as stationary phases in first and second dimension respectively) (620 vs 560). Such a result is essentially due to the high degree of orthogonality between RPLC and SFC which may balance for lesser peak efficiency obtained with SFC as second dimension. Finally, even though current limitations in SFC instrumentation (i.e. large extra-column volume, large dwell volume, no ultra-high pressure) can be critical at the moment for on-line 2D-separations, RPLCxSFC appears to be a promising alternative to RPLCxRPLC for the separation of complex samples of neutral compounds.

1. Introduction

Over the last decades, on-line comprehensive two-dimensional liquid chromatography (LCxLC) has grown significantly in many application fields [1–4]. Liquid chromatography provides a wide variety of separation modes including Reversed Phase Liquid Chromatography (RPLC), Normal Phase Liquid Chromatography (NPLC), Size Exclusion Chromatography (SEC), Ion Exchange Chromatography (IEC) and Hydrophilic Interaction Liquid Chromatography (HILIC). On-line coupling two of these different techniques *via* an appropriate interface may produce a separation system capable of generating a very high effective peak capacity in a reasonable analysis time while avoiding sample loss and/or sample contamination [5].

To maximize the potential of a two-dimensional system, one of the key problems is to find orthogonal conditions between the two dimensions in order to obtain a separation that uses the largest possible fraction (γ) of the separation space [6]. In this regard, NPLCxRPLC was shown to be very attractive for the separation of pharmaceutical compounds [7]. However, in spite of a lower degree of orthogonality, RPLCxRPLC has often been preferred to avoid peak deterioration associated with the incompatibility of the mobile phase of first dimension with that of second dimension (stronger eluting power or immiscibility) [2,8,9] and finally to obtain an

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Table 1
Physical and chemical properties of some bio-oil representative compounds.

	Compound	Chemical family	Molecular Formula	MW (g/mol)	log *P*
1	α-angelica lactone	Lactone	$C_5H_6O_2$	98.10	0.236
2	2-phenylethanol	Alcohol	$C_8H_{10}O$	122.16	1.504
3	5-methylfurfural	Furan	$C_6H_6O_2$	110.11	0.670
4	Phenol	Phenol	C_6H_6O	94.11	1.540
5	o-cresol	Phenol	C_7H_8O	108.14	1.962
6	m-cresol	Phenol	C_7H_8O	108.14	2.043
7	2,4,6-trimethylphenol	Phenol	$C_9H_{12}O$	136.19	2.935
8	α-hydroxycumene	Phenol	$C_9H_{12}O$	136.19	2.861
9	guaiacol	Guaiacol	$C_7H_8O_2$	124.14	1.341
10	syringol	Syringol	$C_8H_{10}O_3$	154.16	1.218
11	1-indanone	Enone	C_9H_8O	132.16	1.419
12	anisole	Aromatic ether	C_7H_8O	108.14	2.170

interesting sample peak capacity for the overall comprehensive system.

Bio-oil samples are mainly composed of small neutral compounds. Two very recent papers [10,11] presented successful separation of aqueous bio-oil extracts by on-line RPLCxRPLC with a percentage of retention space coverage close to 50% only, the retention space being delimited by the retention times of the least and the most retained compounds in both dimension. NPLCxRPLC could be a possible solution to increase the utilized portion of the available space. In this work, we experiment another option which consists in coupling RPLC to supercritical fluid chromatography (RPLCxSFC). West and Lesellier showed that polar stationary phases in SFC tend to behave as in NPLC [12]. Slightly polar stationary phases were also found to be attractive with SFC mobile phases as recently reported in a study which compared their use in SFC and RPLC [13]. On-line SFCxRPLC was investigated by François et al. [14] for the separation of fatty acids in fish oils and compared to on-line RPLCxRPLC for the separation of the same sample. 92% of the separation space was occupied in SFCxRPLC vs 55% in RPLCxRPLC. However, SFCxRPLC arrangement needed a particular interface composed of two two-position/ten-port switching valves equipped with two loops packed with octadecyl silica allowing both the depressurization of the supercritical fluid and the trapping and focusing of the analytes after an addition of water to the first dimension eluent and before the transfer to the second dimension. The potential of RPLCxSFC was highlighted by Stevenson et al. [15] in off-line mode. On-line RPLCxSFC has never been investigated yet. Here we describe our development of on-line RPLCxSFC for the separation of aromatic neutral compounds and an aqueous extract of bio-oil. With a liquid eluent in the first dimension, the interface between the two dimensions is simpler than that used in SFCxRPLC and similar to that used in RPLCxRPLC. Moreover, in the second dimension, the low viscosity of SFC mobile phase allows very fast analysis, which is of prime importance to increase peak capacity in on-line two-dimensional separations. This paper deals with the choice of SFC stationary phase, the study of phenomena resulting from the injection of a polar sample solvent into a supercritical mobile phase and the experimental and instrumental aspects related to the interface. Finally, a comparison between RPLCxSFC and RPLCxRPLC separations of the same aqueous bio-oil extract is proposed in terms of orthogonality, effective peak capacity and sensitivity.

2. Experimental

2.1. Material and reagents

Acetonitrile (ACN) (HPLC grade), methanol (MeOH) and acetone were purchased of HPLC grade from Sigma–Aldrich (Steinheim, Germany). Water was obtained from an Elga water purification system (Veolia water STI, Le Plessis Robinson, France). Pressurized liquid CO_2 3.0 grade (99.9%) was obtained from Air Liquide (Pierre Bénite, France).

The synthetic sample for RPLCxSFC experiments was composed of different compounds known to be representative of those found in bio-oil aqueous samples [10]. It contains α-hydroxycumene, phenol, 2,4,6-trimethylphenol, 1-indanone, syringol, angelica lactone, m-cresol, o-cresol, anisole, guaiacol, 5-methylfurfural and phenylethanol. They were dissolved in water/ACN 85/15 v/v at the concentration of 50 mg/L. Physical properties of these twelve compounds are reported in Table 1. The compounds were either obtained from Sigma–Aldrich or provided by IFP Energies nouvelles (Solaize, France). The bio-oil aqueous sample was provided by IFP Energies nouvelles.

2.2. Columns

Four columns from Waters (Milford, MA, USA) were used under SFC conditions: Acquity UPC^2 BEH-2EP, Acquity UPC^2 BEH, Acquity UPC^2 CSH Fluoro-Phenyl (all 50 mm × 2.1 mm, 1.7 μm, 100 Å) and Acquity UPC^2 HSS C18 (50 mm × 2.1 mm, 1.8 μm, 130 Å). Three columns were used under RPLC conditions: XBridge C18 (50 mm × 1.0 mm, 3.5 μm) from Waters, Hypercarb (100 mm × 1 mm, 5 μm) from Thermo Scientific (Cheshire, UK) and Acquity CSH Phenyl-Hexyl (50 mm × 2.1 mm, 1.7 μm) from Waters.

2.3. Apparatus

2.3.1. 1D-SFC system

Waters Acquity UPC^2 system was equipped with a binary solvent delivery pump, a 250 μL mixing chamber, an autosampler with a 10 μL loop, two column ovens compatible with temperature up to 90 °C and including two 6-channel column selection valves, a UV detector with a 8 μL flow-cell withstanding a pressure up to 414 bar and a backpressure regulator (BPR). The maximum allowable flow rate is 4 mL/min. The maximum allowable pressure is 414 bar for flow-rates below 3.25 mL/min. The maximum allowable pressure linearly decreases to 293 bar when the flow rate increases from 3.25 mL/min to 4 mL/min. Data acquisition was performed by Empower software (Waters). The extra-column volume and extra-column variance were measured under liquid chromatographic conditions. They were equal to 83 μL and 132 μL^2 respectively. The system dwell volume was estimated at 300 μL (see Section 2.4.1).

2.3.2. RPLCxRPLC system

The RPLCxRPLC system was a 2D-IClass liquid chromatograph from Waters. This instrument includes two high-pressure binary solvent delivery pumps, an autosampler with a flow-through needle of 15 μL, a column manager composed of two column ovens with an allowed maximum temperature of 90 °C and two 6-port high pressure two-position valves acting as interface between the two separation dimensions, a UV detector and a diode array